



Considerations and Recommendations to Standard Testing with *Daphnia magna*

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TU066 Considerations and Recommendations to Standard Testing with *Daphnia magna*.

D. Cupi, DTU (Technical University of Denmark) / Department of Environmental Engineering; **A. Baun**, Technical University of Denmark / Department of Environmental Engineering. Standard testing procedures for aquatic organisms, as developed by OECD and ISO, have been developed for chemicals that generally dissolve in aqueous solution. However, many nanomaterials will not dissolve in media and incubation durations used in standard ecotoxicity tests. Therefore, modifications of current testing procedures may be warranted. Here, we focus on highlighting limitations to *Daphnia magna* immobilization testing, and proposing modifications to ensure the procedures are fit to the type of testing being conducted. In the current study, six reference nanoparticles [TiO₂ (NM-104), Ag (NM-300K), CeO₂ (NM-212), ZnO (NM-110), ZnO (NM-111), and SiO₂ (NM-200)] were employed to test acute toxicity on freshwater crustacean *Daphnia magna*. It was seen from the results that the toxicity of nanoparticles to *Daphnia magna* ranked in the following order Ag > ZnO (NM-110) > ZnO (NM-111) > CeO₂ > TiO₂ > SiO₂. In general, nanoparticles in the powdered form were more difficult to suspend in MilliQ water, especially those present in a hydrophobic state, which had a tendency to float. For the latter, 0.1% ethanol was used to create a wetting effect and achieve a paste form, prior to suspension. It was noticed that the physico-chemical properties/size distribution of the stock suspensions changed over time, therefore it is recommended that stock suspensions are prepared fresh shortly before testing. Another issue that affected size distribution was the presence of ions in media (ionic strength), and sonication procedure (bath vs. probe sonicator). Therefore, dispersion of different types of nanoparticles should be considered on case-by-case basis. Additions of environmentally relevant substances such as natural organic matter (NOM) to different concentrations of Ag NPs decreased toxicity. This effect was seen starting from 10 mg/L humic acid, and more efficiently at concentrations of 50 mg/L and 100 mg/L. While addition of NOM may assist in more controlled dispersions with less degree of agglomeration, it may also influence the sensitivity of the test systems. Hence, further research is needed to disclose the influence of allowing addition of NOM in standard *daphnia* tests.